

24098-168

App-Beamer/Dad 9/12/99

EXPRESSION OF TNF-LIKE PROTEIN

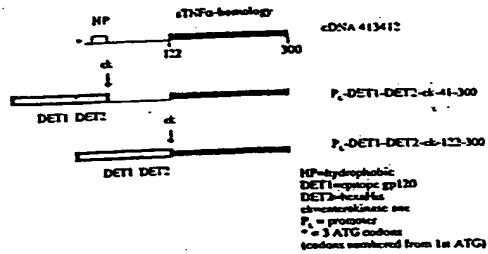
WGS clear 41412 - HTPANOS

Obligates.

- Express as fusion protein in *E. coli*—to be used for raising antibodies
 - Express in soluble form in *E. coli* or other systems—to be used for receptor binding and activity assays

Accomplishments:

- Plasmid DNA from HGS strain (purified by K. B. Tan) was sequenced. The DNA sequence in the open reading frame agreed with that reported by HGS. There was a 1 bp difference upstream of the first of 3 closely spaced in-frame ATG codons, which makes it more likely that the first ATG is the translation initiator codon (**TTCA**^{ATG}G in HGS sequence is **ATCA**^{ATG}G in SB sequence; the latter is in good agreement with Kozaik consensus upstream sequence)
 - Two fusion constructs being made for *E. coli* expression; both have the potential for release of soluble protein following enterokinase digestion.



```

DEFINITION of: TGAJ_HUMAN check: 696 from: 3 to: 232
ID TGAJ_HUMAN STANDARD: 0072 233 AA
PRIMER: 5'-TTTCTTAA-3'
OT: 31-JUL-1996 (REL. 01, CREATED)
OT: 21-JUL-1996 (REL. 02, LAST AMPLIFICATION REPORTED)
OT: 01-JUN-1998 (REL. 03, LAST AMPLIFICATION UPDATE)
OT: 01-JUN-1998 (REL. 04, LAST AMPLIFICATION UPDATE)
THERMOCYCLER CYCLE: PCR (TGAJ-ALPHAS) (SEQUENCING) - - -
seq: 613412; seq: 613412; check: 5774 from: 34 to: 1445
THERMOCYCLER CYCLE: PCR (TGAJ-ALPHAS) (SEQUENCING) - - -
OT: 01-JUN-1998 (REL. 04, LAST AMPLIFICATION UPDATE)
LOCUS 613412
DEFINITION TGAJ Human (Gene associated)
ACCESSION G32412
LIBRARY HTWU_Human_Prefraction_Tumor - - -
Symbol comparison table: Conservation: 100% (Sequence Data, Standard) Sequence: 100%
CapCheck: 1254

Cap weight: 7.000 Average match: 8.340
Length weight: 0.250 Average mismatch: -0.394
Quality: 75.9 Length: 233
Statistic: 0.233 Genes: 2
Number: 1 Average Identikit: 31.814

```

SITE OF START
- DCT1-DCT2 ETC

SCIENTIST SIGNATURE

Eduard Pfeil

Ruben EXHIBIT #32

No. 24098-168

Appleton/Del 97295

EXPRESSION OF TNF-LIKE PROTEIN

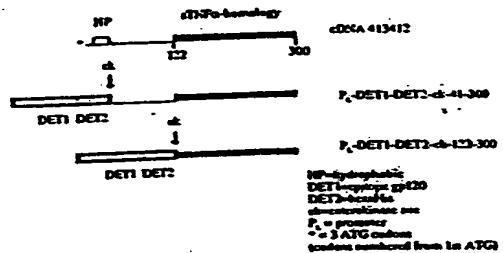
HGS door 413412 - HTPANes

Chickens:

- Express as fusion protein in *E. coli*—to be used for raising antibodies
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Accomplishments:

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 - Two fusion constructs being made for *E. coli* expression: both have the potential for release of soluble protein following enterokinase digestion.



5. БИОПСИЯ АДЕНОМЫ ПРСН В СЛУЧАЕ ПРИВЛЕЧЕНИЯ К РАБОТЕ 24
6. 24
7. ОБРАЩЕНИЯ ПОСЛЕ ПРИВЛЕЧЕНИЯ К РАБОТЕ 27
8. ВСЕГДА ПРЕДЛАГАЮЩИЕ ПРОФЕССИОНАЛИЗМ 283
9. 283
10. ПРИЧИНЫ ПОДДЕРЖАНИЯ ПРОФЕССИОНАЛИЗМА 287
11. 287
12. ПРИЧИНЫ ПОДДЕРЖАНИЯ ПРОФЕССИОНАЛИЗМА 293
13. 293
14. ПРИЧИНЫ ПОДДЕРЖАНИЯ ПРОФЕССИОНАЛИЗМА 297
15. 297
16. ПРИЧИНЫ ПОДДЕРЖАНИЯ ПРОФЕССИОНАЛИЗМА 299
17. 299
18. ПРИЧИНЫ ПОДДЕРЖАНИЯ ПРОФЕССИОНАЛИЗМА 303
19. 303

SITE OF STREET
DET1-DET2 EX
122-300

SCIENTIST SIGNATURE *Edward J. H. F.*

Ruben EXHIBIT 2032
Ruben v. Wiley et al.
Interference No. 105,077
RX 2032

No. 24098-168

Appelbaum/Del 9/12/93

EXPRESSION OF TNF-LIKE PROTEIN

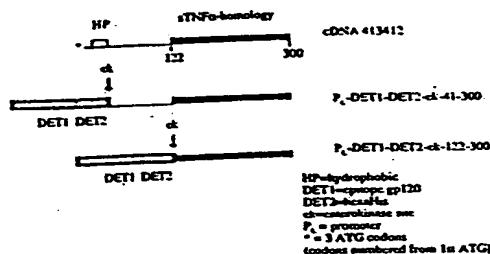
HGS clone 413412 = HTPAN08

Objectives:

- Express as fusion protein in E. coli—to be used for raising antibodies
- Express in soluble form in E. coli or other systems—to be used for receptor binding and activity assays

Accomplishments:

- Plasmid DNA from HGS strain (purified by K. B. Tan) was sequenced.
The DNA sequence in the open reading frame agreed with that reported by HGS.
There was a 1 bp difference upstream of the first of 3 closely spaced in-frame ATG codons, which makes it more likely that the first ATG is the translation initiator codon (TTCAATGG in HGS sequence is ATCAATGG in SB sequence; the latter is in good agreement with Kozak consensus upstream sequence)
- Two fusion constructs being made for E. coli expression; both have the potential for release of soluble protein following enterokinase digestion.



ACTIVITY OF: TNFα Human clone: 413412 from 1 to 233
ID: TNP110001 STANDARD: PEST: 233 AA.
AC: M03773
CT: 21-JUL-1993 (INC: 01, CREATED)
CT: 21-JUL-1993 (INC: 01, LAST SUBMISSION UPLOADED)
CT: 01-JUN-1994 (INC: 29, LAST AMENDMENT UPLOADED)
GE: TUMOR NECROSIS FACTOR PRECURSOR (TNF-ALPHA) (CACHECTIN)

acc: 413412.Pep checks 2940 from 1 to 279

TRANSLATE of: 413412 checks 8756 from 50 to 1645

generated symbols 1 to 520.

LOCUS: 413412

DEFINITION: PEST signal peptide removed

VERSION: 413412

LIBRARY: HTPA Human Protein Data

Symbol comparison table: Conservation:(Coppere.Data.Bundest)Biology.Chr

CompCheck: 1254

Cap weight: 7.000 Average match: 0.340

Length weight: 0.250 Average mismatch: 0.394

Quality: 75.0 Length: 233

Score: 0.333 Gap: 2

Percent Similarity: 35.394 Average Identifiers: 24.034

Time: Human 413412.Pep July 31, 1993 23:06

7/31/93

5' ENTHOVLKEDLPLVQPCNCLFLSPLVWVATVQLLL 54

61 QDCAQFLKEDLPLVQPCNCLFLSPLVWVATVQLLL 107

122 VEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 160

139 LVEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 177

146 EPEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 197

153 VLEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 219

160 VEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 226

177 LVEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 243

200 PQLCQFLKEDLPLVQPCNCLFLSPLVWVATVQLLL 272

217 LVEVQFPLVPLVQPCNCLFLSPLVWVATVQLLL 279

SCIENTIST SIGNATURE: *Edward J. Appelbaum*

SITE OF START
DET1-DET2 EK
122-300

DATE: 11/16/93

partner: James W. Shewell
Date: 9/19/93

No. 24098-169

SYNTHETIC DNA REQUEST FORM

Please read these instructions before you write your oligos:

1. Please write all oligo sequences from 5' to 3' direction.
2. Write sequences in capital letters using a pen (Not pencil).
3. You can request more than one oligo on a same page.
4. Write your name, project, department and phone number.
5. Write your sequences as 5' ATG - CCC - CAA - TTC - ACA - TTT 3'
6. If you need specialty oligos (Biosyn, Amico, Bsolid, Dromabell, Thermo etc), please write them accordingly.

OLIGO SEQUENCES:

1. 5' CGG - GAT - CCT - CGA - CGA - CGA - CAA AGA - GCT -
GAA - GCA - GAT - GLA - CGA 3' SB6686
2. 5' CGG - GAT - CCT - CGA - CGA - CGA - CAA AGT - AGC - AGC -
TUA - CAT - AAG - TGG 3' SB6687
3. ~~CGG - CCT - CGA~~
4. GLT - CTA - CAT - TAG - CCA - ACT - AAA - AAG - GLL SB6688

Requestor: EDWARD DUL

Project: Chemocore

Department: GCS

Telephone Number: 7708

Indicate for what purpose you are requesting these oligos:

PCR primers Sequencing primers Linker Primers
 Antibodies Gene construction Affinity Columns
Human Growth Factor, Lys 7200 or Joyce Stahl, Ext 1687 for help.

Please let me know if I have any questions - R. Dul

from 3/7/95

HGS clone 413 417 (ATG 343) was sequenced in its entirety and was in agreement with the HGS sequence. This was the template for the pcr reactions of the TNF beta plasmid.

S1, S2 designate pcr reactions to obtain DRT1, DRT2 & K-HI-300.

RK, 122-300 designate pcr reactions to obtain DRT1, DRT2

SCIENTIST SIGNATURE

Edward Dul

DATE 11/17/95

partner Joann O'Rourke Feb 7, 1996

24098-170

Arg-343-2-3/1

1. $\text{Ar}^2 \text{SbBr}_3 / \text{SDAD} \text{H}_2$

SG 6686	(48-min)	1.38 ug/l	95 min
SG 6687	(45-min)	1.65 ug/l	107 min
SG 6688	(27-min)	1.40 ug/l	157 min

	ATC 343	ATC 345	ATC 346
ATC 573 (avg.)	1.0	1.0	8.4
SB 6696	5.0	-	-
SB 6687	-	-	1.0
10 x TAC Polycarbonate	11.0	6.0	6.0
Scrum 100 L	8.64	-	9.40
1.5 M XTT	8.0	-	1.0
TAC Pol.	1.0	1.0	7.04
H ₂ O	13.0	-	-
SB 6688	1.0	-	-

ATG 343
S-1

14

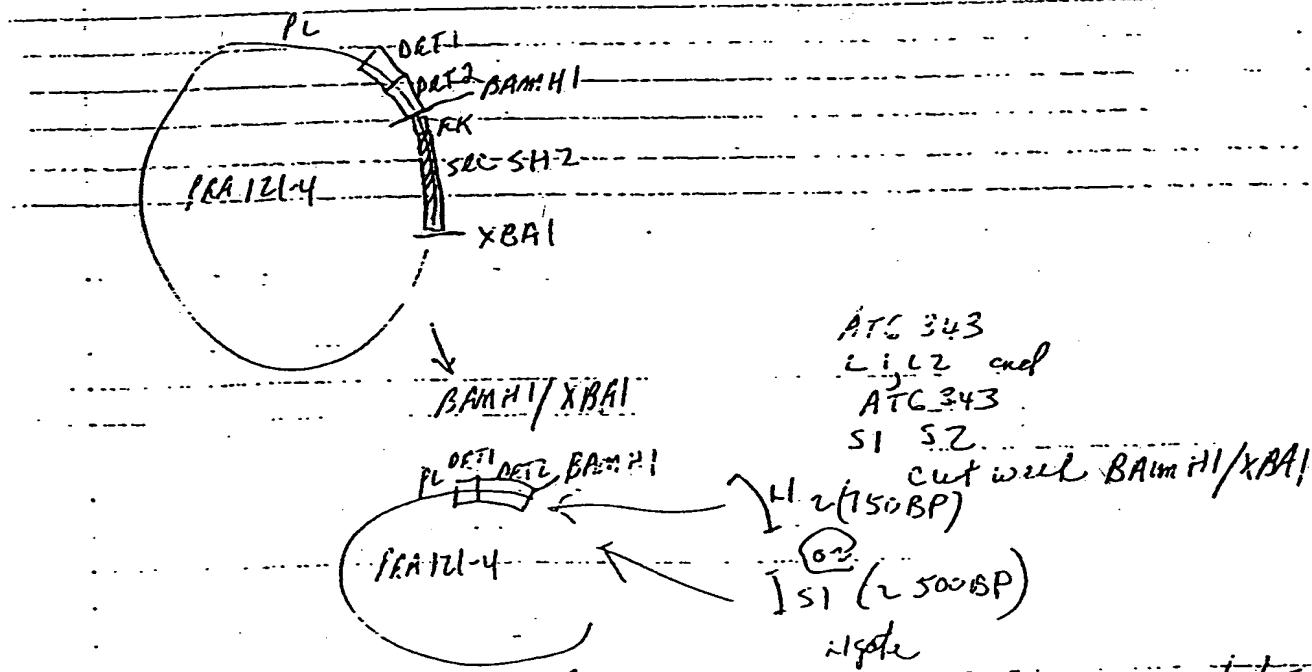
A 16 343
S-7

-

卷之三

Cut choice for fragments will Bant II / XBA

Piglet pRA121-4 with BAmH1/XBAI



SCIENTIST SIGNATURE *Richard Sefcik*

DATE 1/17/95

entres Flores & Poulard 5-29 1976

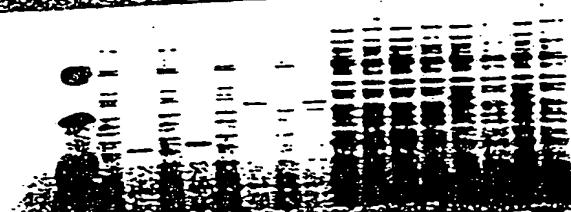
No. 24098-171



Sequene of candidates L1 F3, S2 #3 are as expected

micro-algal constructs in LW 14

PHOTOGRAPH BY DR. ROBERT WILSON



INDUCTIONS (LW 14)
PER DAY DATA FOR TNFLIKE

	0'	41
L-1-3	.586	1.5
L-2-2	.638	1.63
S-1-3	.614	1.67
S-2-3	.606	1.63

normalize cells to 3D
including soft tissue
removed

Whole cells

ecm mass 130g
water 14g

Fractions

ecm mass 200g
water 6g

DATE 4/17/95

SCIENTIST SIGNATURE

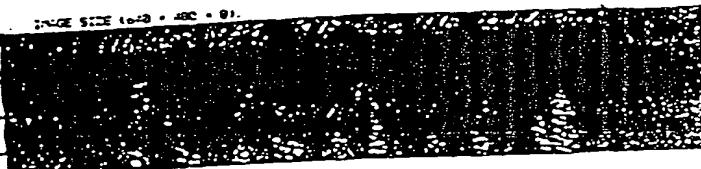
Robert Wilson

Microalgae Project Feb 9, 1995

24098-172

whole
cells

STRATAGENE EXCLUSIVELY 03 10/16/95 14:28:03



Run western Blot of above sample using 178.1 (DRII antisera)

Band on coomassie lights up using DRII Antisera (178.1)

SCIENTIST SIGNATURE

Richard Duf

DATE 11/17/95

return to me AT 10:00 AM 12/1/95

No. 24098-180

TNF-like gene (AT6-343) now called TL2-L (long)
and TL2-S (short)

Chris Jones purified a liter induction of
TL2-L (Both DNA sequences of TL2-DAT1-DAT2
forms have been confirmed as expected)

Material was highly insoluble and C. had to
solubilize it in water and dilute it off.

Obtained ~ 50 mg material in 65 ml

Injected into rabbits for Antisera

To determine solubility ^{at low Temp} of various forms. DAT1-DAT2-L & K
TL2 (both forms L and S) in GE 698 with Tryptophan
induction at 28° and 32°

		0'	4'
①	GE 698 TL2-L1 (28°)	.460	1.19
②	GE 698 TL2-S2 (28°)	.436	1.07
④	GE 698 TL2-S2 (32°)	.407	1.16

whole cell pellets / sediment fractions

* Have obtained from HGS 2 Antisera to TL2 (AT52)
Call HGS TL2-1 and HGS TL2-2

Obtained pre bleed from TL2 rabbit sera (both
pre bleed and 1st bleed) - called CK9A and CK9B

I was unable to see expression at well in this
strain as I was able to see well in AT58 or AT170

Run Western's (crude cells/10μl - 50 μl/10 spot 30 μl
100ng DAT1-DAT2-TL2 purified by Chris Jones
1/2000 dilutions of RAB

SCIENTIST SIGNATURE

DATE

Edward Karp

Western Nov. 21, 1996
Ed Karp Nov. 9, 1996

No. 24098-181

western Blot showed immunofixa of purified TC2 with
Ball amy anti-sea (cR9R) band much more faintly than with
with the HGS anti-sea TC2-7

SI 698
TCL
induction
commercial
STAIN

NH₂ Terminal of CKR-13 purified from *Microsphaera* permits expression of CKB-13 in *S. cerevisiae* (CPMK).

SCIENTIST SIGNATURE

DATE 4/1/96

Walter J.us Alford Feb 9, 1996

GLC99 induction of pCA DCT1 DCT2 TC2

overnight OD⁵⁵⁰ growth

L1 - 28°C - 3.5

S2 - 28°C - 3.1

L1 - 32°C - 4.8

S2 - 32°C - 4.6

Dilute each ON in T induction media

	VOL OF N	VOL IN D medium	OD ⁵⁵⁰ DILUTED PLATE
L1 - 28°	1.5	2.5 ml	.269
S2 - 28°	2	.. "	.221
L1 - 32°	1	.. "	.258
S2 - 32°	1	.. "	.244

1 hr 15 min later

2' 4' after addition of 100 μl/ml lysozyme

(1) L1 - 28°	530	1.97
(2) S2 - 28°	463	2.0
(3) L1 - 32°	547	2.26
(4) S2 - 32°	445	2.14

normalize whole cells in lysis Buffer

set aside cells for sonication - normalize them in

Buffer A + lysozyme

(1 hr - 30 min)

+ sonicate

spin microfuge - 30 min

SCIENTIST SIGNATURE

Kejun Wang

DATE 2/8/96

Entered: Jane Abowd Feb 91 996

Run 15% Precast gel of TC2 induction, (coomassie whole cell
Sal/sal fractions Western Blot 20 μl/lane each sample)

1:2500 dilution TC2 antisera (CK9B Blot 3)
1:5000 dilution anti-Rabbit



gel is over loaded
and pre Antisera is
not diluted enough

some encouraging results
(1) Antisera recognises

short form of TC2
in Supernatant at
28°C and possibly
32°C

TC2 western must be
run loading less sample and diluting primary antisera more.

Injet RABBITS CK9A CK9B wull OCT1 OCT2 TC2 long
Injet rabbits CK9A CK9B wull CCA2 peptide

B 13

DO TRANSFECTON of MTALII CCA2 long #5 into S2 cells

PMTALII CCA2 long #5	150 ng/1	250
pcD vector	90 ng/1	1.5 -

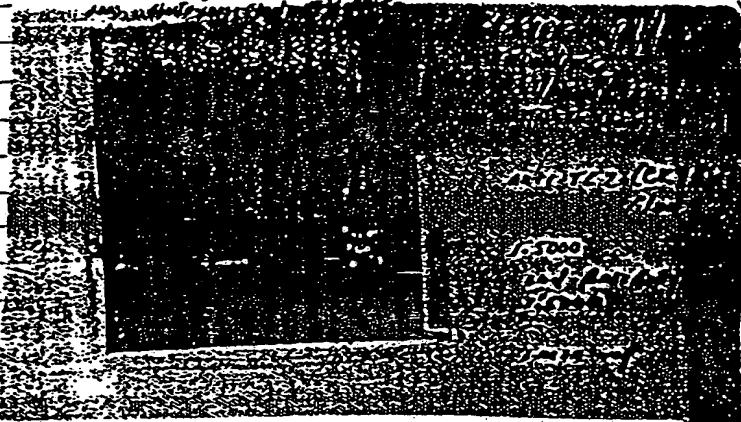
SCIENTIST SIGNATURE

Ed Ifail

DATE 3/5/96

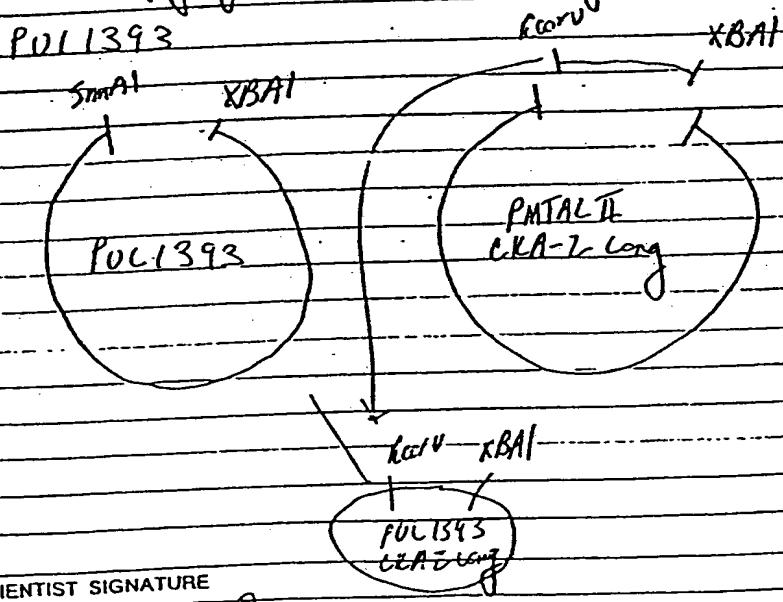
24098-194

Re-run, Western Blot of T1.2 induction) using half lane sample
and antisera at 1:500 dilution



15 DNA sequence of pMTALII CKA-2 long is as expected

Strategy of CKA-2 long cloning is Bacteriophage "vector"



SCIENTIST SIGNATURE

teffler

DATE 3/5/96

No. 24095-196

yield of EHAZ-long hair (YRA) and outer (RGA)

323

Setup liston

4 1393

H 1393

6 1395 long

unl

401393 (long hair) 50g/p 4ml

1.5 long (long hair) 10g/p 6ml

1.5 short (short hair) 10g/p 6ml

S. x Bell. ligase

Ligase

ml

ml

ml

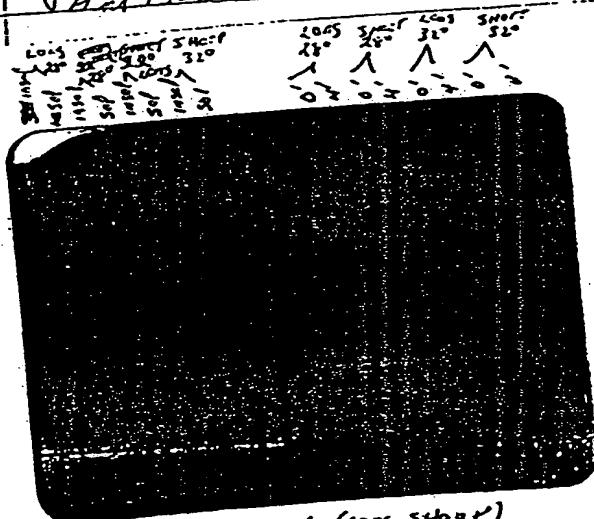
ml

ml

16°C 0/11

LR 2525. Tissue from in ~~the~~ DMSO

Run concave gel of sol/insol fractions GI 698 induction
of DEI1 DEI2 TC2 (pg 191). Also replot western using Act I
Act II Sua.

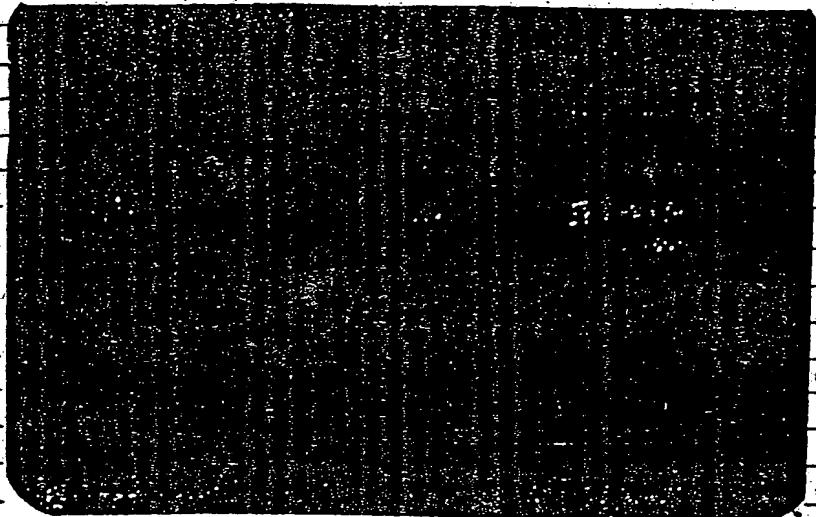


SCIENTIST SIGNATURE

R. J. Puf

DATE 3/5/94

No. 24098-197



26 Coomassie and Crutia Blot using Anti ACT 1 showed
Solubility of ACT1 ACT2 Tc2 short

On 1 liter induction of ACT1 ACT2 ACT Tc2 (concentrated) for
Sachdeva's Purification

OD⁶⁰⁰ of overnight (28°C)

Tc2-long - 2.2

Tc1 short 2.15

(28°C) Dilute 0/N 1:10 (50ml / 450 ml induction medium)

OD⁶⁰⁰ at time of dilution

OD⁶⁰⁰ - Addition of tryptone Eluent (ml/hr)

1 Tc2 short (second)	.191	.410	2.0
2 " "	.237	.456	1.86
3 Tc2 long	.776	.414	1.82
4 "	.734	.410	1.84

SCIENTIST SIGNATURE

G. D. Flug

DATE 3/5/96

No. 24098-198

3/28

DETI DRTZ TLC 49 scale (500A)

STROBOSCOPIC EXPOSURE 1/1000 sec. = 61(95)

Long long shot shot

28°C

IMAGE SIZE 1600 x 400 = 640.

INTERGRATE PERIOD IS 0.09 SECONDS OR 2 FRAMES

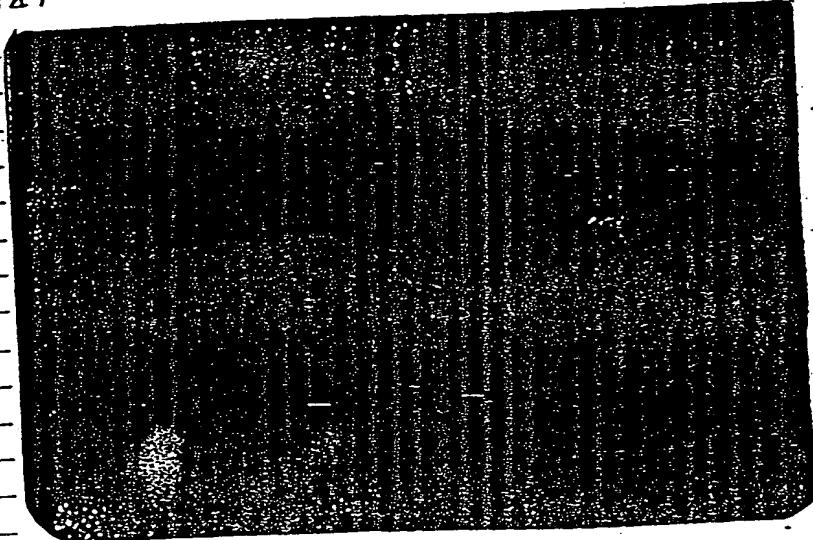
IMAGE CREATED ON SUN 3D 1/12/91 10:12 AM

0 4' 0 4' 0 4' 0' 4' 20 mm



Save some samples for sol/sensal studies

Western Blot - Probe w/ Ab (Anti-DET - 178.1) and (Anti-TLC
CK90)



SCIENTIST SIGNATURE *Kellie*

DATE 3/5/96

No. 24098-199

material (GLC - Start) small peaks soluble wth DMSO and some
Turn over to SLD Fisher

29 More colonies present on pull 1393 CERAC long than Victor
Alcon

SCIENTIST SIGNATURE

Ed Gaff

DATE 3/5/96

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